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Established Cells

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【Inventor】

【Address or Residence】
3-501, 3-banchi, Mitsugamimine 1-chome,
Taihaku-ku, Sendai-shi, Miyagi-ken

【Name】 Kenichi HOSOYA

【Inventor】

【Address or Residence】
2-1-5, Akashiminami, Izumi-ku, Sendai-shi,
Miyagi-ken

【Name】 Tetsuya TERASAKI

【Inventor】

【Address or Residence】
1672-1-719, Imafuku, Kawagoe-shi,
Saitama-ken

【Name】 Masatsugu UEDA

【Inventor】

【Address or Residence】
5-3-10-402, Hachiman, Aoba-ku, Sendai-shi,

Miyagi-ken

【Name】 Masuo OBINATA

【Applicant】

【Code Number】 395007255

【Name】 YS New Technology Institute Inc.

【Attorney】

【Code Number】 100090941

【Name】 Seiya FUJINO

【Teleohone】 3226-6671

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JP10-296139

【Document Name】 Specification

【Title of the Invention】 ESTABLISHED CELLS

【Claims】

【Claim 1】 An established cell derived from choroid plexus epithelial cells, which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of $Na^+$ -$K^+$ ATPase in the apical side.

【Claim 2】 The established cell according to claim 1, having a deposition number of FERM BP-6508.

【Claim 3】 A method of establishing an immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of $Na^+$ -$K^+$ ATPase in the apical side, the method comprising treating choroid plexus epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells to obtain immortalized cells to obtain immortalized cells.

【Claim 4】 The method of establishing an immortalized cell according to claim 3, wherein the transgenic animal is a rat.

【Claim 5】 An established cell which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter in the

cell membrane, and when cultured in a monolayer, shows the localization of $Na^+$ -$K^+$ ATPase in the apical side, which is obtained by treating choroid plexus epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.

【Detailed Description of the Invention】

【0001】

【Technical Field where the Invention Belongs】

The present invention also relates to established cells derived from choroid plexus epithelial cells of the transgenic animal. The established cells derived from choroid plexus epithelial cells of the present invention are useful for studying nutrition metabolism in the brain, studying permeation of drugs into the brain, and investigating the metabolism or permeation protection mechanism of substances in the cerebrospinal system. These cells are therefore useful in screening drugs regarding the safety and efficacy thereof, and developing methods for diagnosing and treating diseases relating to nutrition metabolism disorders and homeostatic functional disorders of the brain on cellular level studies.

【0002】

【Background Art】

Conventionally, tests for the assessment of safety and efficacy of drugs have mainly been conducted using animals. However, to avoid use of a large number of animals from the viewpoint of animal right, test technologies using cultured

cells for in-vitro assessment of safety and efficacy of drugs are used on a practical level. For example, a technique of first testing using primary culture cells collected from living tissues or established culture cells which can infinitely proliferate, and then testing using animals is employed. The primary culture cells can initially proliferate very well, but the proliferation gradually declines as the subculture advances, and finally cells die out. This phenomenon is called cellular senescence. Furthermore, in addition to the fear that the characteristics of primary culture cells may differ each time they are collected from living tissues, the primary culture cells are said to change the characteristics as the subculture advances. Particularly, when the multiplication rate is very slow or when the cells are derived from a small organ, it is very difficult to obtain a sufficient amount of the primary culture cells for test.

【0003】

On the other hand, established culture cell which have acquired the capability of infinitely proliferating during subcultures of the primary culture cells can maintain stable characteristics. However, most of these cells no longer have part or all of the forms and functions possessed by the cells when they were in a living body. Therefore, it is difficult for such established cells to precisely reflect the original characteristics which the cell lines exhibited in the tissues from which they have been derived.

【0004】

In view of this situation, establishment of immortalized

cells which can continuously maintain an active proliferation capability possessed by the primary culture cells without losing the characteristics inherently possessed by the cells during subculture, has been tried by transforming the cells by introducing oncogenes such as ras and c-myc, E1A gene of adenovirus, large T-antigen gene of SV40 virus, HPV16 gene of human papillomavirus, and the like. Such immortalized cells which are derived from some organs already lose several functions at the time of introducing oncogenes or large T-antigen genes after preparation of a primary culture cell. Thus, acquisition of immortalized cells in the stringent meaning of holding an original function has been difficult. Preparing a primary culture cell and acquiring a cell line has been very difficult, particularly when the multiplication rate is very slow or when the cells are derived from a small organ.

【0005】

To overcome these problems, a method of establishing immortalized cells by applying a recently developed transgenic technology to individual animals has been proposed. Instead of introducing oncogenes or large T-antigen genes into individual cells, according to this method, transgenic animals into which these genes have been introduced in chromosomes in a stable manner are prepared. Then, a primary culture cell is prepared from an organ of these animals which possesses the oncogenes or large T-antigen genes in the cells at the time of development of the individuals. The primary culture cells is subcultured to establish immortalized cells. In particular, immortalized cells are easily available from organs of

transgenic mice into which a large T-antigen gene of a temperature sensitive mutant tsA58 of SV40 has been introduced.The immortalized cells are very useful because proliferation of the resulting cells and expression of the differentiation character can be managed by changing the temperature (Noble M. et al. (1995) Transgenic Research 4, 215-225; Obinata M. (1997) Genes to Cells 2, 235-244) . Rats having a body weight about ten times that of mice are advantageous for preparing cells used for the establishment of cells from various organs, particularly for preparing a cell line originating from small organs such as retinal capillary endothelial cells, because primary culture cells can be easily obtained by separating organs. Therefore, transgenic rats into which a large T-antigen gene of a temperature sensitive mutant tsA58 of SV40 has been introduced, which are useful for establishing immortalized cells due to easy availability from various organs and the capability of controlling the proliferation of the resulting cells and expression of the differentiation character by changing temperatures, had already been produced.

【0006】

On the other hand, in research investigating the effect and mechanism of nerve drugs on the blood-cerebrospinal fluid barrier mechanism, a method of using a primary culture cell of choroid plexus epithelial cells in place of animal tests is being developed in view of animal right. In this instance, because it is difficult to constantly obtain a sufficient amount of culture cells for the test from small animals,

effective cell lines usable in place of such culture cells have been strongly desired.

【0007】

【Problems to be Solved by the Invention】

In view of this situation, the present inventors have conducted extensive studies and, as a result, have established immortalized cells by separating an epithelial cell line from the choroid plexus of brain of transgenic rats into which immortalizing genes have been introduced. An object of the present invention is therefore to provide established cells derived from choroid plexus epithelial cells, capable of expressing a temperature sensitive SV40 large T-antigen gene, showing localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transport carriers in the cell membrane, and when cultured in a monolayer, showing the localization of $Na^+$ -$K^+$ ATPase in the apical side.

A further object of the present invention is to provide a method of establishing such immortalized cells using a large T-antigen gene of the SV40 temperature sensitive mutant tsA58.

【0008】

【Means to Solve the Problems】

The present invention relates to established cells derived from choroid plexus epithelial cells. Specifically, the present invention relates to established cells expressing a temperature sensitive SV40 large T-antigen gene, showing localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transport carriers in the cell membrane, and when cultured in a monolayer, showing the localization of $Na^+$ -$K^+$ ATPase in the apical side. FERM

BP-6508 can be given as such established cells.

The present invention also relates to a method of establishing immortalized cells comprising treating the choroid plexus tissues of a transgenic animal into which a large T-antigen gene of a temperature sensitive mutant tsA58 of SV40 has been introduced with protease, selecting the cells exhibiting an epithelial cell-like/paving stone-like form from the resulting cells, and subculturing such cells. The rat can be given as an example of such a transgenic animal.

Furthermore, the present invention relates to the established cells obtained using such a method.

Due to the capability of forming tight junction among cells when cultured in a mono-layer on a porous flat membrane and the capability of reconstructing the blood-cerebrospinal fluid barrier having a inside-and-outside polarity in vitro, the established cells obtained by the present invention are useful for studying nutrition metabolism of the brain, studying permeation of drugs into the brain, and investigating the metabolism or permeation protection mechanism of substances in the cerebrospinal system. These cells are therefore useful in screening drugs regarding the safety and efficacy thereof, and developing a method for diagnosing and treating diseases relating to nutrition metabolism disorders and homeostatic functional disorders of the brain on cellular level studies.

【0009】

【Embodiments of the Invention】

The transgenic rat using in the present invention into

which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced can be obtained as follows. Specifically, a whole genome DNA of tsA58ori(-)-2 which is produced from a large T-antigen gene of a temperature sensitive mutant tsA58 of SV40, for example, with deletion of the SV40 ori (replication origin), is linearized using a restriction endonuclease BamHI, and introduced into pBR322 to obtain a plasmid pSVtsA58ori(-)-2 (Ohno T. et al., Cytotechnology 7, 165-172 (1991)). The plasmid is amplified in Escherichia coli in a large amount according to a conventional method. The plasmid thus obtained is cut with a restriction endonuclease BamHI to eliminate a vector region. Because the DNA (5,240 bp) having a large T-antigen gene of tsA58 thus obtained has a promoter of the large T-antigen gene therein, a rat into which the DNA is introduced expresses this gene (the large T-antigen gene of tsA58) in all somatic cells. Next, the resulting DNA is introduced into totipotent cells of rats in accordance with a conventional method to prepare transgenic rats having a temperature sensitive large T-antigen gene in all cells. As a totipotent cell, ES cells having totipotency can be given in addition to fertilized ova and early embryos. A microinjection method, electropolation method, liposome method, calcium phosphate method, and the like can be used for introducing DNA into such ova and cultured cells. Furthermore, the present gene can be introduced into ova by transplanting a nucleus of cultured cells, into which a desired gene of the present invention has been introduced, in enucleation unfertilized ova and initializing the ova (nuclear

transplantation). However, as far as the efficiency of obtaining a transgenic rat is concerned, a transgenic rat having a large T-antigen gene of tsA58 incorporated into chromosomes of cells of each tissue at the time of development of individuals can be efficiently obtained by producing ova through microinjection of the gene of the present invention into male pronucleus of the pronucleus fertilized ova, transplanting the ova into the oviduct of an foster mother to obtain offspring, and selecting the offspring having the injected gene, thereby stably obtaining individuals into which the gene of the present invention has been incorporated.

【0010】

Immortalized cells can be prepared by extracting cells (primary cells) from organs of gene-introduced rats thus obtained, and repeating subculture of the cells according to a conventional method. The resulting cells have the capability of permanently proliferating at 33-37°C and terminating the proliferation at 39°C. The brain of this rat is taken out to collect choroid plexus. The choroid plexus cut into pieces is treated with trypsin/EDTA to disperse cells. After terminating the enzymatic reaction by the addition of a culture solution containing serum, the cells are collected by centrifugation and dispersed in a culturesolution. The procedures of centrifugation and dispersion are repeated to wash the cells. The cells thus obtained are dispersed in a culturesolution, inoculated on a culture plate, and incubated at 33°C. After subculturing three generations, colonies are formed. Colonies exhibiting a paving stone-like form inherent to epithelial

cell and a comparatively fast growth rate are isolated from the surrounding cells using a penicillin cup. This procedure is repeated twice to isolate the cell lines originating from a single cell. The cell lines obtained are subjected to immunostaining to confirm localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter on the cell membrane by using a confocal laser scanning microscopy, whereby the cells are identified. The resulting cell lines express a large T-antigen, maintain excellent proliferating activity after 50 generation subculture at 33°C, and exhibit expression of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter. In particular, when the cells are cultured in a monolayer, $Na^+$ -$K^+$ ATPase which is present on the basolateral membrane side (a serous membrane side) in other epithelial cells, is locally present in the apical side of the cell membrane.

【0011】

【Example】

The present invention will now be described in more detail by way of examples, which are given for the purpose of explanation and should not be construed as limiting the present invention.

【0012】

【Example 1】

Preparation of transgenic rat

A transgenic rat carrying DNA of an SV40 temperature sensitive mutant tsA58 was prepared according to the following method.

①Preparation of a gene to be introduced

DNA of SV40 temperature sensitive mutant tsA58 was used for microinjection. The genome DNA of tsA58 was linearized using a restriction endonuclease BamHI and introduced into the BamH site of pBR322 to convert the Sfi I sequence to the SacII sequence, thereby obtaining a DNA clone pSVtsA58 ori(-)-2 with deletion of the SV40 ori site (replication origin)(See Ohno T. et al., Cytotechnology 7, 165-172 (1991), Figure 1). The DNA was prepared from the pSVtsA58 ori(-)-2 according to a conventional method. Specifically, the pSVtsA58 ori(-)-2 of plasmid DNA obtained by amplification in Escherichia coli. was digested using a restriction endonucleases BamHI (made by Takara Shuzo Co., Ltd.) and DNA (Linear DNA fragment) of tsA58 with a length of 5240 bp separated the vector region by agarose gel electrophoresis (1% gel; Boeringer company)were cut out from the gel. The gel was dissolved by agarase treatment (0.6 unit/100 mg gel: Agarase; made by Boeringer Co.). DNA was recovered by phenol-chloroform treatment and ethanol precipitation treatment. The recovered and purified DNA was dissolved in a TE buffer (10 mM Tris-HCl containing 1 mM EDTA, pH 7.6) to obtain a purified DNA solution with a concentration of 170 μg/mL. The DNA solution was diluted with a buffer (10 mM Tris-HCl containing 0.1 mM EDTA, pH 7.6) to a concentration of 5 μg/mL to prepare a DNA solution for microinjection. The resulting DNA solution was stored at -20°C until use for microinjection.

【0013】

②Preparation of transgenic rat

Preparation of transgenic rat Microinjection of the DNA

solution prepared in ① above to the rat fertilized ova at pronucleus stage was carried out according to the following procedures. Sexually mature Wistar rats, aged eight weeks, were kept in a condition of a 12 hour light-and-shade cycle (light hours: 4:00-16:00) at 23±2°C and RH 55±5%. The estrous cycle of female rats was observed by vaginal smear to select the hormonal treating day. A pregnant-mare serum gonadotropic hormone (pregnant mare serum gonadotropin; PMSG, manufactured by Nippon Zenyaka Co.) was intraperitoneally administered at a dose of 150 IU/kg to female rats. After 48 hours, 75 IU/kg of human chorionic gonadotropic hormone (human chorionic gonadotropin; hCG, manufactured by Sankyo Zoki Co.) was administered thereby effecting superovulation treatment. The female and male rats were mated by being together in a cage. The fertilized ova at pronucleous stage were collected by oviduct perfusion at 32 hours after the hCG administration. A mKRB solution (Toyoda Y. and Chang M.C., J. Reprod. Fertil., 36, 9-22 (1974)) was used for the oviduct perfusion and incubation of ova. The collected (fertilized) ova were treated by an enzyme in an mKRB solution containing 0.1% hyaluronidase (Hyaluronidase Type I-S, made by Sigma Co.) at 37°C for 5 minutes to remove cumulus cells. After washing three times with the mKRB solution to remove the enzyme, the fertilized ova were stored in a $CO_2$ incubator (5% $CO_2$-95% air, 37°C, saturated humidity) until DNA microinjection. A DNA solution was microinjected into the male pronucleus of the rat (fertilized) ova thus prepared. 228 ova after microinjection were transplanted in nine recipients (foster mothers) and 80

pups were obtained. The integration of the microinjected DNA was analyzed with DNA prepared from tails of the rats immediately after weaning by the PCR method (primers used: tsA58-1A, 5'-TCCTAATGTGCAGTCAGGTG-3' (corresponds to 1365-1384 sites), tsA58-1B, 5'-ATGACGAGCTTTGGCACTTG-3' (corresponds to 1571-1590 sites)). As a result, 20 rats (6 male, 8 female, and 6 unknown sexuality) were identified to have the gene introduced. Among these rats, 11 transgenic rat lines (male lines: #07-2, #07-5, #09-6, #12-3, #19-5, female lines: #09-7, #11-6, #12-5, #12-7, #18-5, #19-8) which survived as long as 12 weeks after elapse of the sexual maturation period were obtained. These $G_0$ generation transgenic rats were mated with Wistar rats and established 2 lines of male founders (#07-2, #07-5) and 3 lines of female founders (#09-7, #11-6, #19-8), by confirming that the genes was transferred to next generation.

【0014】

【Example 2】

Isolation of choroid plexus epithelial cells

In a clean bench, the brain was collected from one transgenic rat carring a large T-antigen gene of SV40 temperature sensitive mutant tsA58 obtained in Example 1. The choroid plexus from the inner wall of the right and left ventriculus lateralis through the upper wall of the third ventricle of the brain was collected and thoroughly washed with PBS. The tissue was cut into pieces with a volume of 1-2 $mm^3$ in 2 mL of ice-cooled PBS. The tissue pieces were suspended into 1 mL of a 10X trypsin/EDTA solution (0.5%

Trypsin, 0.53 mM EDTA; manufactured by Gibco BRL) to digest by the enzyme treatment (37°C, 20 minutes). The tissue pieces were dispersed by gently stirring from time to time. The resulting cells were washed with a culture medium (DEME solution containing 10% FCS, 100 U/mL benzylpenicillin potassium, and 100 μ/mL streptomycin sulfate). The cells were dispersed in 2 mL of the culture solution and inoculated in a 35mmö culture dish (Falcon, manufactured by Becton Dickinson Co.) and incubated (primary culture) at 33°C in a $CO_2$ incubator (5% $CO_2$-95% air, saturated humidity). Subculture was carried out at an interval of about one week using a trypsin/EDTA solution (0.05% Trypsin, 0.53 mM EDTA; manufactured by Gibco BRL) while replacing the medium twice a week. After subculture three times, $10^2$-$10^3$ cells were inoculated in a 10 cmö culture dish and incubated in a $CO_2$ incubator at 33°C to form colonies. After 7-10 days while replacing the medium twice a week, the colonies consisting of cells having a paving stone-like form inherent to epithelial cells which exhibit a comparatively fast growth rate were isolated from the surrounding cells using a penicillin cup. The cells which were obtained were again inoculated in a 10 cmö culture dish and incubated at 33°C in a $CO_2$ incubator to form colonies. Colonies exhibiting a comparatively fast growth rate were isolated using a penicillin cup to obtain five lines of cells (TR-CSFB1, TR-CSFB2, TR-CSFB3, TR-CSFB4, TR-CSFB5).

TR-CSFB3 was deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and

Industries. The deposition number is FERM BP-6508.

【0015】

【Example 3】

Confirmation of large T-antigen proteins

Large T-antigen proteins in the five cell lines obtained in Example 2 were examined by the Western Blotting method (Experimental Medicine Separate Volume, Biotechnology Manual UP Series, "Cancer research protocol by the molecular biological approach", pages 108-115, YODOSHA Publishing Co., 1995). The five cell lines (the $10^{th}$ generation) were cultured in a 90 mmö culture dishes until saturation. The collected cells were solubilized using 1 mL of 3% SDS-PBS (pH 7.4) and unsolubilized fractions were removed by centrifugation (10,000 rpm, 10 minutes), and then the total amount of proteins was determined by the Bradford method (using the protein assay kit II of BIO-RAD Co.). The proteins were separated by the SDS polyacrylamide gel electrophoresis in the amount of 20 μg each and transferred onto nitrocellulose membranes. The nitrocellulose membranes blocked by a 3% skimmed milk solution were reacted with an anti-SV40 large T-antigen mouse antibody (DP02-C, CALBIOCHEM Co.), as a primary antibody, and a HRP labeled anti-mouse IgG antibody A (Amersham Co.), as a secondary antibody, to detect the reactions specific to large T-antigen proteins using the ECL Western Blotting detection system (RPN2106M1, a product of Amersham Co.). The results are shown in Table 1. As a result, the large T-antigen proteins were detected in all five cell lines obtained.

【0016】

【Table 1】

| Cells | TR-CSFB1 | TR-CSFB2 | TR-CSFB3 | TR-CSFB4 | TR-CSFB5 |
|---|---|---|---|---|---|
| T-Antigen | + | + | + | + | + |

【0017】

【Example 4】

Confirmation of $Na^+$ -$K^+$ ATPase and GLUT-1 transport carrier

The cells obtained were cultured in a mono-layer and expression of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter on the cell membrane was confirmed by confocal laser scanning microscopy observation of immunologically stained cells. The TR-CSFB3 cells obtained in Example 2 were cultured on a collagen coated cover glass of a 35 mmö dish (Falcon). After removal of the culture solution, the cells were washed with PBS, then 4 mL of a fixative (PBS containing 3% paraformaldehyde and 2% sucrose) was added. After allowing to stand at room temperature for 15 minutes, the cells were thoroughly washed with PBS. 2 mL of a blocking solution (Block Ace, manufactured by Dainippon Pharmaceutical Co., Ltd.) was added and the mixture was allowed to stand for one hour at 37°C to effect blocking, followed by the reaction with a primary antibody (anti $Na^+$ -$K^+$ ATPase β2 rabbit antibody, a product of UBI, or anti-GLUT-1 rabbit antibody, a product of Chemicon) for one hour at room temperature. The resulting product was washed four times with PBS and reacted with a secondary antibody (FITC labeled anti-rabbit IgG, a product of Capel) for one hour at room temperature, followed by washing with PBS four times. Finally, labeled cells were sealed with a glycerol sealing solution (a 90% glycerol solution in PBS containing 0.1% (v/v) of Perma Fluor (a product

of Lipshaw)). The cover glass periphery was sealed with a mani cure. A confocal laser scanning microscopy (CLSM; Zwiss LSM 410, manufactured by Zwiss) was used for the observation. As a resul t, expression of $Na^+$ -$K^+$ ATPase (Figure 1) and GLUT-1 were detect ed in TR-CSFB3 cells. In particular, $Na^+$ -$K^+$ ATPase which is pre sent on the basolateral membrane side (a serous membrane side) i n other epithelial cells was seen to be locally present in the a pical side of the cell membrane, the cell lines obtained were id entified to be choroid plexus epithelial cell lines. The same r esults were obtained with other cells.

【0018】

【Example 5】

Confirmation of proline transport capability

The concentration dependency of the resulting cells on the L-proline transport was examined to determine the L-proline transport capability. This was compared with the reported values of L-proline transport capability in the choroid plexus, thereby confirming that the resulting cell lines have functions as the choroid plexus epithelial cells. Specifically, TR-CSFB3 cells obatined in Example 2 were inoculated in a 24-well cell culture plate at a concentration of $3x10^5$ /well/mL and incubated for 24 hours at 33°C in a $CO_2$ incubator to be the cells confluent. After removal of the medium by aspiration, the cells were washed with an uptake buffer (1) previously heated at 37°C (prepared from a solution which contains 122 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4 \cdot 7H_2O$, 0.4 mM $K_2HPO_4$, 10 mM Hepes, and 25 mM $NaHCO_3$ by bubbling 5% $CO_2$-95% $O_2$ into the solution for 20 minutes and

adjusting the pH of the resulting solution to 7.4 with NaOH). The uptake buffer Solutions (1) containing proline at different concentrations were prepared by adding non-labeled L-proline to the solutions to make final concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20 mM. After the uptake reaction for 30 minutes and washing three times with PBS, 1 mL of PBS containing 1% Triton X-100 was added and the mixture was allowed to stand overnight to solubilize the cells. The radioactivity was measured using a liquid scintillation counter (LS-6500 made by Beckmann Co.). In addition, the amount of proteins was determined using a protein assay kit manufactured by Bio-Rad Co. Using the plot formula for the uptake rate vs. the L-proline concentration ($V = Vmax \times [S]/(Km + [S])$, wherein Vmax indicates a maximum velocity constant, Km indicates the Michaelis constant, and [s] is a substrate concentration), the Km and the Vmax for L-proline uptake were analyzed using the non-linear minimum square program (Yamaoka K. et al. (1981) J. Pharmacobio-Dyn., 4, 879-885). The results are shown in Figure 2. As a result, it was confirmed that the uptake of L-proline ([$^3$H]-L-proline) was concentration-dependent, the Km was 1.5 mM, and the Vmax was 2.4 nmol/min/mg protein. The value for Km as determined was similar to the Km value reported on rabbit choroid plexus of 1.1 mM (Coben L.A. et al. (1972) Brain Res., 30, 67-82). This confirms that the resulting cells possess the function of choroid plexus epithelial cell line.

【0019】

【Example 6】

Inhibition of proline active transport by choline and ouabain

The L-proline uptake into the isolated choroid plexus is dependent on $Na^+$. Therefore, the $Na^+$ dependency of the L-proline uptake by the cells obtained was confirmed, and then the cells were confirmed to have functions as the choroid plexus epithelial cells in the same way as in Example 5. However, because the experiment must be carried out under $Na^+$-free conditions, the uptake buffer (1) being replaced all $Na^+$ in the buffer with coline were used. For the confirmation of the effect of ouabain, the uptake buffer (1) containing a tracer to which 1 mM of ouabain was added was used (because ouabain is an inhibitor of $Na^+$ -$K^+$ ATPase, the concentration gradient of $Na^+$ is disappeared.). Both reactions were carried out for 30 minutes. The results are shown in Figure 3. It was also confirmed that L-proline uptake was inhibited as much as 98% under $Na^+$-free conditions. It was confirmed that 56% of L-proline uptake was inhibited by 1 mM ouabain. As a result, the L-proline uptake of TR-CSFB3 cells was confirmed to be $Na^+$-dependent. This confirms that the resulting cells possess the function of choroid plexus epithelial cell line.

【0020】

【Effects of the Invention】

Established cells derived from choroid plexus epithelial cells are provided. The cells express a temperature sensitive SV40 large T-antigen gene, show localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, show the localization of $Na^+$ -$K^+$ ATPase in the apical side. Also provided is a method of establishing

immortalized cells, comprising treating choroid plexus tissues of a transgenic animal into which a large T-antigen gene of an SV40 temperature sensitive mutant tsA58 was produced by protease treatment.

Due to the capability of forming tight junctions among cells when cultured in a mono-layer on a porous flat membrane and the capability of reconstructing the blood-cerebrospinal fluid barrier with a inside-and-outside polarity in vitro, the established cells are useful for studying nutrition metabolism in the brain, studying permeation of drugs into the brain, and investigating the metabolism or permeation protection mechanism of substances in the cerebrospinal system. These cells are therefore useful in screening drugs regarding the safety and efficacy thereof, and developing a method for diagnosing and treating diseases relating to nutrition metabolism disorders and homeostatic functional disorders of the brain in cellular level studies.

【Brief Description of the Drawings】

【figure 1】

Figure 1 shows confocal laser scanning microscopy of $Na^+$ -$K^+$ ATPase of the established cell (TR-CSFB3) obtained in Example 4 of the present invention.

The upper photograph is a microscopic photograph of a plan view of the cell wherein $Na^+$ -$K^+$ ATPase and GLUT-1 are seen to be expressed. The lower photograph is a microscopic photograph of a cross section view of the cell wherein $Na^+$ -$K^+$ ATPase are seen localized in apical side.

【figure 2】

Figure 2 shows the proline active transport capability of the established cell obtained in Example 5 of the present invention.

【figure 3】

Figure 3 shows interference of the proline active transport capability of the established cell obtained in Example 6 of the present invention by choline and ouabain.

JP 10-296139

【Document Name】 Abstract

【Abstract】

【Problems】 To provide established cells.

【Means to be solved】

An established cell derived from choroid plexus epithelial cells, which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of $Na^+$ -$K^+$ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of $Na^+$ -$K^+$ ATPase in the apical side.

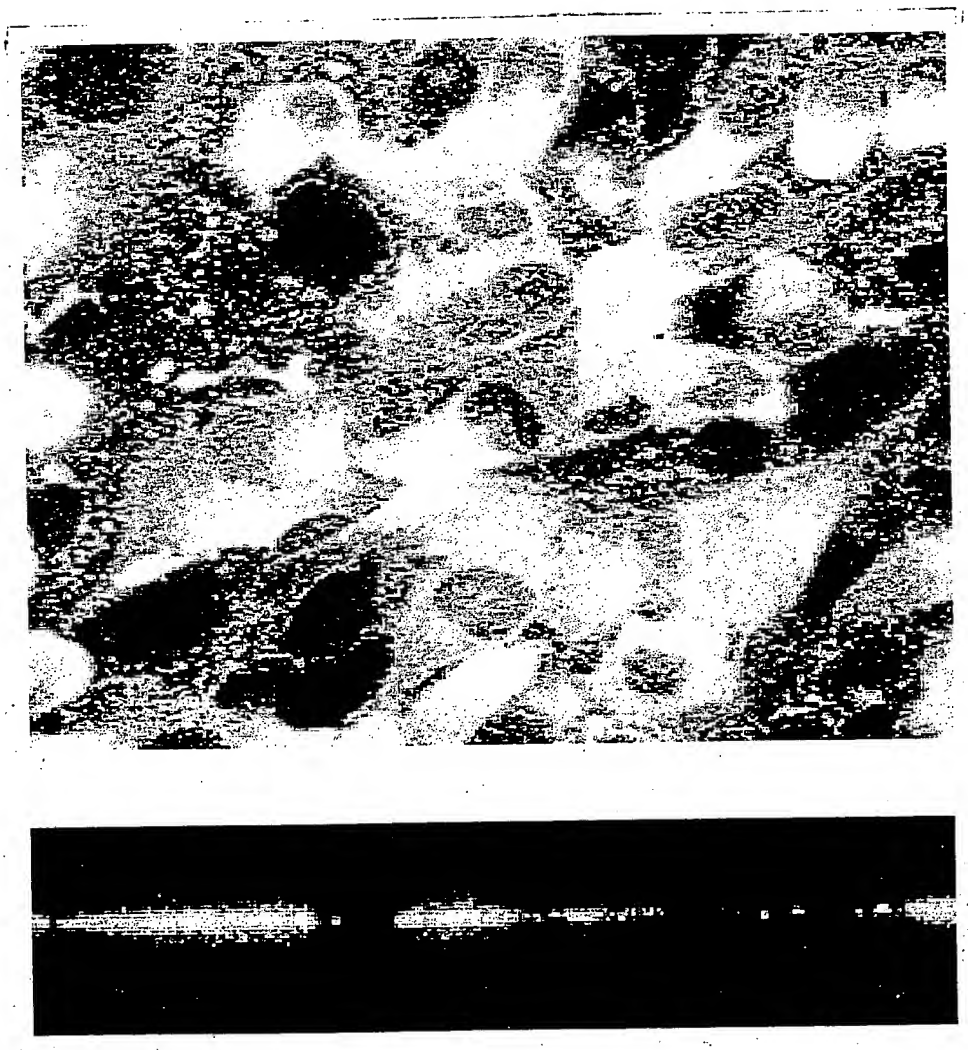
A method of establishing an immortalized cell comprising treating choroid plexus epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.

【Effect】

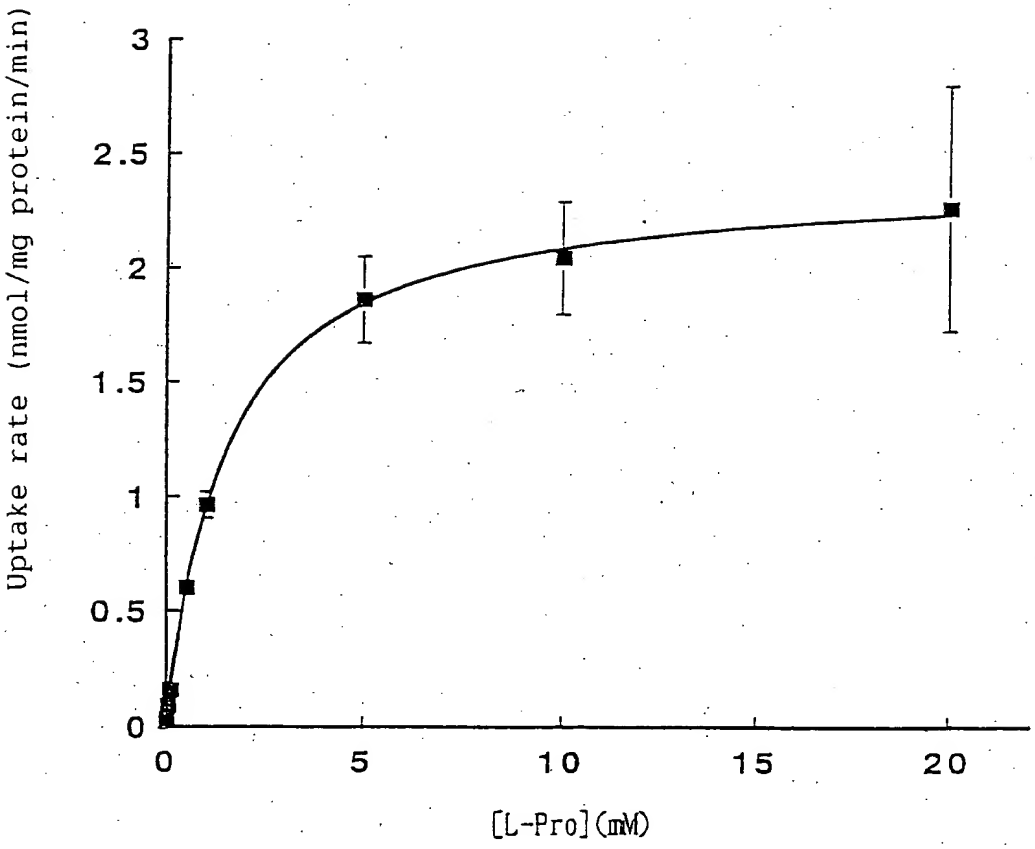
These cells are useful in screening drugs regarding the safety and efficacy thereof, and developing a method for diagnosing and treating diseases relating to nutrition metabolism disorders and homeostatic functional disorders of the brain in cellular level studies.

【Drawing】 Non

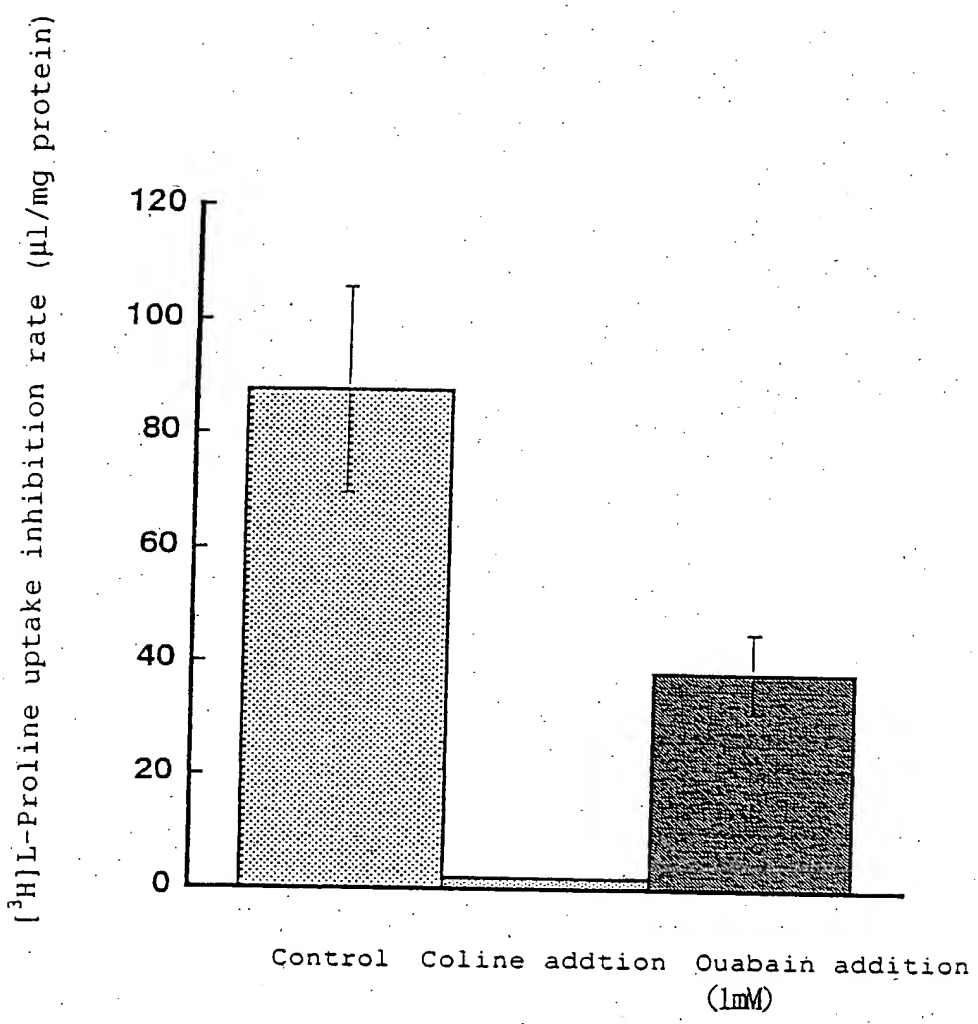
【 Figure 1 】



【 Figure 2 】


Uptake rate (nmol/mg protein/min)
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
[L-Pro](mM)

【 Figure 3 】


[3H]L-Proline uptake inhibition rate (μl/mg protein)
120
100
80
60
40
20
0
Control
Coline addtion
Ouabain addition
(1mM)

整理番号 SNMFP98379



国際様式 INTERNATIONAL FORM

[特許手続上の微生物の寄託の国際的承認に関するブダペスト条約]

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

下記国際寄託当局によって規則7．1に従い発行される。

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page.

# 原寄託についての受託証

寄託者

氏名（名称） 株式会社 ワイエスニューテクノロジー研究所 取締役研究所長 上田 正次 殿

あて名 〒 329－0512
栃木県下都賀郡石橋町大字下石橋字花林 5 19番地

| 微生物の表示 | |
|---|---|
| （寄託者が付した識別のための表示）<br>TR－CSFB3 | （受託番号）<br>FERM BP－ 6508 |

2．科学的性質及び分類学上の位置

1欄の微生物には、次の事項を記載した文書が添付されていた。

□ 科学的性質
■ 分類学上の位置

3．受領及び受託

本国際寄託当局は、平成 10 年 9 月 18 日（原寄託日）に受領した1欄の微生物を受託する。

4．移管請求の受領

本国際寄託当局は、 年 月 日（原寄託日）に1欄の微生物を受領した。
そして、 年 月 日 に原寄託よりブダペスト条約に基づく寄託への移管請求を受領した。

5．国際寄託当局

通商産業省工業技術院生命工学工業技術研究所

名称： National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology

所長 大箸 信一郎

Dr. Shin-ichiro Ohashi Director-General

あて名： 日本国茨城県つくば市東1丁目1番3号（郵便番号305－8566）
1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken 305-8566, JAPAN

平成10年（1998） 9月18日